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Local and systemic cytokine profiles in non-severe and severe community-acquired pneumonia

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Material and methods

Study design

Patients who fulfilled the following criteria were enrolled in the study: 1) age 18 years or over; 2) clinical presentation of an acute illness with one or more of the following symptoms suggesting CAP: presence of fever ($t \geq 38^{\circ}C$), dyspnoea, coughing (with or without expectoration of sputum), chest pain; and 3) presence of new consolidation(s) on the chest radiograph. Patients were excluded from the study if one of the following criteria applied: nursing home residency or hospital admission in the previous 15 days, presence of immunosuppression (e.g. HIV infection, systemic immunosuppressive agents including oral corticosteroids and chemotherapy), presence of a systemic autoimmune disease, pulmonary tuberculosis, bronchiectasis, malignancy, or the presence of obstruction pneumonia. Ten healthy volunteers matched for age, sex and smoking status and without a history of cardiac or pulmonary disease, malignancy or autoimmune disease served as the control group.

Upon admission, we collected data on age, gender, smoking habits, comorbidities, clinical signs and symptoms, chest radiography, biochemical analysis, microbiological findings, previous antibiotic treatment and time between pneumonia onset and inclusion. Selection of antibiotic treatment was based on national guidelines [1]. The pneumonia severity index (PSI) was also determined upon admission and patients were classified as non-severe CAP patients (PSI classes 1-3), or as severe CAP patients (PSI classes 4 or 5).

Obtaining and processing of BAL and blood samples

After written informed consent and within 24 hours after admission, bronchoalveolar lavage (BAL) fluids were collected with a flexible fibre-optic bronchoscope (Olympus) according to recommended guidelines [2]. The bronchoscope was introduced into the

bronchus corresponding to the most abnormal area on the chest radiography. In patients with diffuse pulmonary infiltrates, the middle lobe or lingular division was chosen. Three aliquots of 20 ml sterile saline were instilled and subsequently gently aspirated. BAL fluids were filtered through a 100µm cell strainer (BD Biosciences), collected on ice and immediately centrifuged at 450x*g* for 10 minutes at 4ºC. Supernatant was stored at -80ºC and analysed in one batch.

Venous blood samples were collected directly prior to the BAL procedure. At days 7 and 30 after admission, another venous blood sample was collected from the patients. Blood samples were centrifuged at 1200x*g* for 10 minutes at 4ºC. Serum was taken, stored at -80ºC and then analysed in a single batch.

Measurement of cytokines in BAL fluid and serum

Cytokine levels in BAL fluids and serum of IL-6, IL-8, IL-10, IL-1β, TNFα, IFNγ, IL-22, IL-17A and IL-4 were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available assays (IL-8 OptEIA Set, BD Biosciences; all other cytokines Ready-Set-Go kits, eBiosciences). The limits of detection in pg/ml were: 2.0 for IL-6, 0.8 for IL-8, 2.0 for IL-10, 4.0 for IL-1β, 4.0 for TNFα, 4.0 for IFNγ, 8.0 for IL-22, 4.0 for IL-17A and 2.0 for IL-4.

Microbiological studies

Prior to initiation of antibiotic therapy, two sets of blood cultures were taken from each patient. In patients with a productive cough sputum samples were obtained upon admission for Gram staining and culture. Furthermore, urine samples were taken for antigen detection of *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1. Gram

stainings and cultures were performed on BAL specimens and, if clinical symptoms suggested, pharyngeal swabs were taken for identification of viral pathogens.

References

1. SWAB The Dutch Working Party on Antibiotic Policy. Guideline on antimicrobial treatment of community-acquired pneumonia (CAP). Amsterdam, 2005.
2. British Thoracic Society Bronchoscopy Guidelines Committee aSoSoCCoBTS. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. *Thorax* 2001: 56 Suppl 1: i1-21.